BULLETIN

OF THE

BROOKLYN ENTOMOLOGICAL SOCIETY

VOL. XXXVI OCTOBER, 1941 NO. 4

THE PREPARATION OF SLIDES OF THE GENITALIA OF LEPIDOPTERA.

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Within the past 4 years I have had numerous requests from students and technicians for a paper dealing with the method employed in the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, in the preparation of the slides of the genitalia of Lepidoptera.

In this paper I am describing in detail the procedure followed and am listing convenient equipment which may be modified according to the wishes of those who follow this method. It is not my object in this paper to discuss other methods, some of which are highly satisfactory, but merely to set forth the method of procedure which has proved satisfactory in the preparation of well over 30,000 slides of genitalia now housed in the Division of Insects of the United States National Museum.

EQUIPMENT.

The equipment used is simple, and falls into two classes, (1) mechanical and (2) chemical. It is assumed, of course, that the operator owns, or has access to, a dissecting microscope and essential accessories.

Suitable implements and other equipment are as follows: Three 4-ounce dropper bottles for reagents; six to twelve 3-inch test tubes, corks, pins, and rack; one 2-ounce glass-stoppered bottle for dye and one 2-ounce jar for balsam. Two types of watch glasses are convenient, the Syracuse watch glass (four) for washing and dissecting, and the small Bureau of Plant Industry watch glass (about ten or twelve) for clearing. Two scalpels with triangular outline and sharp cutting edge, two dissecting needles, two fine hooks, small flexible forceps, and two or three camel's-hair brushes of different small sizes are necessary. If desired, these brushes

may be trimmed to give shorter bristles and truncate ends but the trimming of the brushes is entirely a matter of choice. Both types of brushes are used by Bureau lepidopterists. The above may be supplemented by a small syringe, made from a hypodermic needle and fitted with a rubber bulb from a medicine dropper, used to force extraneous matter from the abdomen. In addition, a suitable supply of hollow-ground microscope slides, cover glasses (18 and 22 mm.), and rings of various thicknesses is essential. The hooks can be made by inserting small insect pins (No. 0 or minuten nadeln) in the ends of small sticks or of small camel's-hair brushes from which the hairs have been removed. The points of the pins are then bent to form the desired hooks. The rings are made of "bakelite" or hard vulcanized fiber, and can be obtained in the form of tubing three-quarters of an inch (19 mm.) in diameter, outside measurement. The tubing must then be cut, with a lathe, into the desired thicknesses. Other types of rings, already prepared, are on the market and can be obtained for a small sum.

The chemicals or reagents used are few, inexpensive, and easily obtained. They consist of an adequate supply of 95% alcohol, xylene (xylol), Canada balsam, oil of cloves, a 0.5% solution of mercurochrome, a 10% solution of potassium hydroxide (KOH) in 50% alcohol, and plenty of water.

The 50% solution of alcohol is used in the preparation of the 10% solution of potassium hydroxide, to increase the wetting power.

The 0.5% solution of mercurochrome, used for staining the tissues, is prepared by dissolving one mercurochrome tablet (4.6 gr.) in 2 ounces of distilled water. It is advisable to use distilled water in the preparation of the stain to eliminate the rare chance of a chemical reaction with the mercurochrome. Under ordinary circumstances, however, ordinary tap water will serve.

The best results in mounting the genitalia will be obtained by thinning the Canada balsam with xylene. Thick, viscid balsam is difficult to handle and slows up the procedure.

Procedure.

In selecting specimens for dissection it is well to follow a few established rules.

(1) Select topotypes (specimens from the type locality) or homotypes (specimens compared with types) when possible, or

(2) Select specimens in good condition to assure correct specific identity, or (3) select a male and a female from the same

locality if the series is small or males and females from different localities, to include all variations of a species, if the series is large.

A word of caution here: Any specimen with the abdomen glued on should be distrusted. Unless such a specimen is marked with a label, indicating that the correct abdomen is attached, it should not be used for genitalic studies.

When the specimen for dissection has been selected, the next step is the preparation of the labels. Two labels are needed, one a small, permanent label (fig. 4) to be attached to the pin on which the specimen is mounted, the other a temporary slip (fig. 2) which is attached to the cork of the test tube containing the soaking abdomen. The first bears the notation that the genitalia are on a slide, the date of preparation, and the slide number; the second, which is discarded after the permanent slide labels are made, shows the essential data to be recorded on the slide labels. This temporary label bears a number, corresponding to that on the label attached to the insect. The number will be transferred to the slide later.

To remove the abdomen of the specimen all that is usually necessary is a slight pressure on the underside which causes it to break off. Occasionally if care is not exercised, the breaking point will come between the meso- and metathorax. This can be avoided by applying counter pressure with a stout dissecting needle, or the point of one of the small scapels, to the suture (dorsal) between the metathorax and the first abdominal segment. Cutting the abdomen posterior to the first abdominal segment is not advised, because characters of important taxonomic value are frequently found on this segment. If, after the dissection of a specimen of a large species, it is found that the first, or subsequent segments, have no characters of taxonomic value, then the abdomen may be cut to reduce the space consumed on the slide. After removal, the abdomen is dropped into one of the test tubes of the 10% KOH, where it is allowed to remain from 2 or 3 (small microlepidoptera) to 24 hours (Geometridae, Phalaenidae, most Rhopalocera, and the majority of the microlepidoptera), depending on the size of the specimen. In some groups (Sphingidae, Bombycidae, etc.) the abdomen should soak for 48 hours.

After the abdomen has soaked long enough to dissolve most of the tissues (except the sclerotized parts) it is removed and placed in a watch glass of water (Syracuse). In this step the extraneous matter is forced from the abdomen, the scales are removed with one of the camel's-hair brushes, and all the excess potassium hydroxide is washed out. At this point the small syringe may be

used to force the undesired material from the abdomen. Then the intersegmental membrane between the eighth abdominal segment if male, or seventh if female, and the genitalia is cut and the latter removed. Any foreign matter remaining in the abdomen or bits of tissue remaining attached to the genitalia are now eliminated. If any scales remain attached to the abdomen they can be removed later in the alcohol wash.

The abdomen and genitalia are now ready for staining. The stain is made by putting a few drops of the stock solution of dye (0.5% solution of mercurochrome) into a watch glass of water. Here the dissected parts are allowed to remain until stained to suit. Care should be taken not to permit the tissues to become too dark. A faint pink color is desirable but no more. The lightly sclerotized parts are more sensitive to the dye than those which are strongly sclerotized. If the genitalia are permitted to remain in the stain too long the membranous areas become dark and many essential details may be obscured. The membranous parts should be stained lightly, however, to eliminate the possibility of their disappearance in the balsam.

After staining, transfer the parts to a watch glass of 95% alcohol to remove excess dye and to dehydrate. In this wash it is possible to remove any remaining scales and to clean up any extraneous matter or ragged edges. If the genitalia are from male specimens they are spread (if symmetrical) at this time.

When the alcohol wash and dehydration have been completed the genitalia and abdomen are removed and placed in one of the small watch glasses containing oil of cloves. The oil will clear and harden the parts so that they will retain the desired shape and position obtained in the preceding alcohol wash. If oil of cloves is not available cedar oil may be substituted.

The genitalia are allowed to remain in the oil until clear and then transferred to a large watch glass of xylol (xylene), where the excess oil is washed off. At this point the aedeagus may be removed (if desired) although this may also be done equally well in the alcohol before clearing in oil.

Now the genitalia are ready for mounting. On a slide, previously cleaned, place a drop or two of the balsam. Into this introduce the genitalia and abdomen, and arrange as indicated in figure 5 if a male, and figure 6 if a female. This arrangement may be modified according to the size of the parts. If small, the genitalia and abdomen can be placed together under a single cover glass, but if large the genitalia should be mounted separately, with the

abdomen at one side (fig. 3). When the genitalia are in position a cover glass is placed over them. Rings (figs. 3, 5, and 6) are used to support the cover glass only when the genitalia are too thick to permit mounting without distortion.

In all cases where large genitalia are mounted it will be necessary to use rings to give sufficient depth to the balsam to accommodate the parts. Rings of various thicknesses will be necessary for these mounts (figs. 5, 6). The genitalia should never be pressed down and flattened, for this always distorts or breaks them.

Uniformity in mounting of the genitalia cannot be too strongly stressed. The position of the various parts of the genitalia of a given group should be the same for purposes of comparison. What applies to one group perhaps will not apply to another. For example: In group "A" (figs. 7, 8) the genitalia may be shown to the best advantage spread (male) and from the ventral side; in group "B" (figs. 10, 14) it may be better to expose them in the lateral view or to dissect them. In the Crambidae (fig. 16) the harpes show to the best advantage dissected and in ventral view, while the tegumen, uncus, and gnathos show to the best advantage from the side. Most of the Phalaenidae (both males and females) may be seen best in ventral view, but not a few require mounting in lateral aspect. The genitalia of most microlepidoptera should be mounted to show the ventral side, but those of male Gelechiidae (fig. 17) and a few others can be observed best usually in lateral or three-quarters aspect.

In figure 24 I have illustrated an example of a preparation in which "drifting" has occurred. To avoid drifting three rules should be observed.

(1) Avoid the use of thick, viscid balsam.

(2) Insure the rapid "flowing on" of the cover glass; if necessary, by touching the edge of the cover glass in xylol.

(3) Do not place slides on edge while fresh.

The genitalia in fresh preparations will always drift if the slide is placed on edge. Slides frequently require several months to harden, if they are thick, so it is always advisable to house them in a horizontal position. Slides may be hardened more rapidly by placing them in an oven or on a radiator. This method of hardening slides, however, often discolors them. It is most desirable to permit hardening to take place slowly.

If drifting does take place in spite of all precautions, the genitalia may be replaced in their proper positions by introducing a minuten

nadeln pin, or a bristle, into the balsam from underneath the cover glass. The mounted parts can then be moved, with the end of the pin or bristle, into the desired positions.

When possible it is advisable to make more than one slide of a species (the more the better) and to mount one or two in different positions from the usual.

No one should be satisfied with anything less than the best slide that it is possible to make. It is not enough that one is able to recognize the parts and make identifications. The slide should be made carefully enough to photograph without distortion so that in the reproduction there will be found a true display of the characters.

In this connection I should like to quote from an editorial by Carl Heinrich (Proc. Ent. Soc. Washington, vol. 26 (1), p. 25, 1924). ". . . We want specimens, and particularly we want specimens with a history. The field workers should supply them. They frequently do not. Two common excuses are offered for this failure: the worker has no time to devote to preserving specimens, or he is not able to prepare them properly—this last more frequently in the case of the smaller Lepidoptera. The first of these is not true. If one can afford time for an experiment, he can afford time to carry it on carefully. The second is no excuse at all. It is a confession. It puts one in the class with the carpenter who cannot drive a nail or use a saw. No entomologist is worthy of the name who cannot make decent preparations—slide or otherwise—of the insects he studies. An untrained schoolgirl can do it with a week's practice. A scientist should be able to do as much."

After mounting, the slides are labeled (fig. 3), the label bearing data corresponding to that on the insect. If a single mount is used the slide should bear two labels, each with half the data shown in figure 3, leaving space for any additional data that one may wish to add later. Each slide is numbered to correspond to the small label attached to the pin at the time of the removal of the abdomen.

In closing, a few remarks on the reproduction of figures of genitalia for publication may be in order.

It will be seen from the illustrations in this paper that photographs are not entirely satisfactory. Specks of dirt in the balsam detract from the appearance of the illustration; more frequently than not the genitalia are too thick to permit focusing on all parts, and thus many details are obscured; the balsam becomes dark with age, thereby clouding the illustration. These are a few of the details that militate against the photographic process.

Line drawings, reproduced as zinc etchings, are the most satisfactory, but their preparation is time-consuming and expensive. The most satisfactory way of illustrating genitalia will probably be found in the combination of photographs of entire mounts (figs. 7, 8, and 11) supplemented by drawings of essential details.

I wish to thank Mr. M. L. Foubert of the Office of Information, United States Department of Agriculture, for his painstaking care in making the illustrations for this paper. Through his knowledge of photography and helpful coöperation it has been possible to obtain photographs emphasizing the good, as well as bad, features where desired.

PLATE I

FIG. 1. Equipment used in the preparation of slides of genitalia.

Plate II.

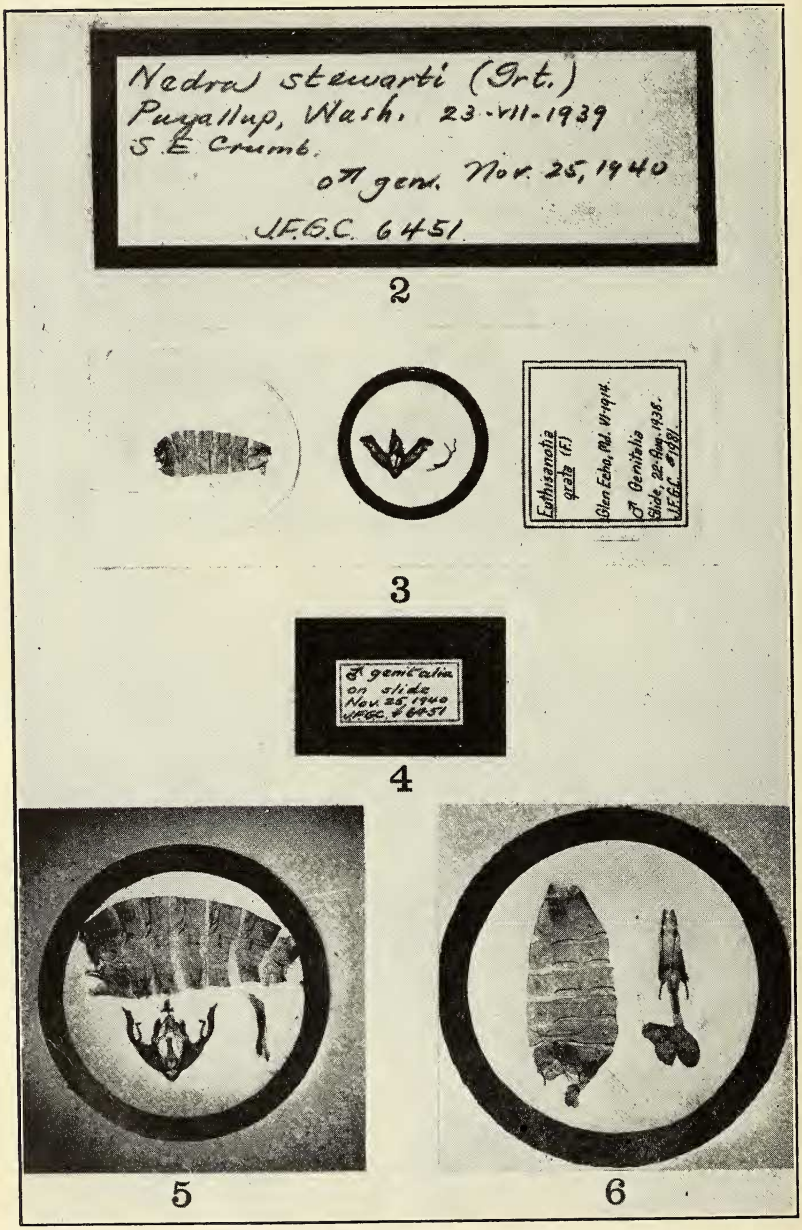

Nedra stewarti (Grt.)
Puyallup, Wash. 23-VII-1939
S. E. Crumb.
♂ gen. Nov. 25, 1940
J.F.G.C. 6451
2
Lethrinanthia
gretta (F.)
Glen Echo, Md. VI-1914.
♂ Genitalia
Slide, 22-Apr.-1935.
J.F.G.C. #6881
3
♂ genitalia
on slide
Nov. 25, 1940
J.F.G.C. # 6451
4
5
6

Plate III.

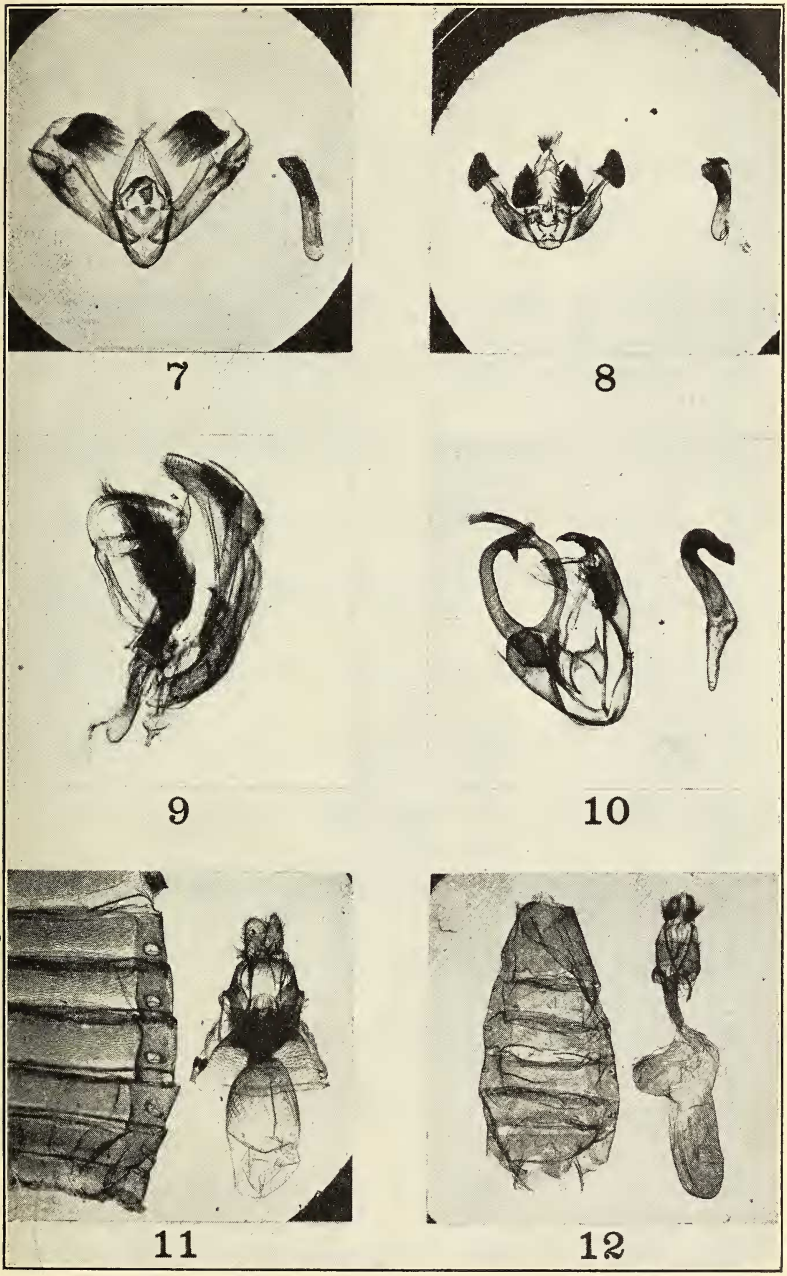


Plate IV.

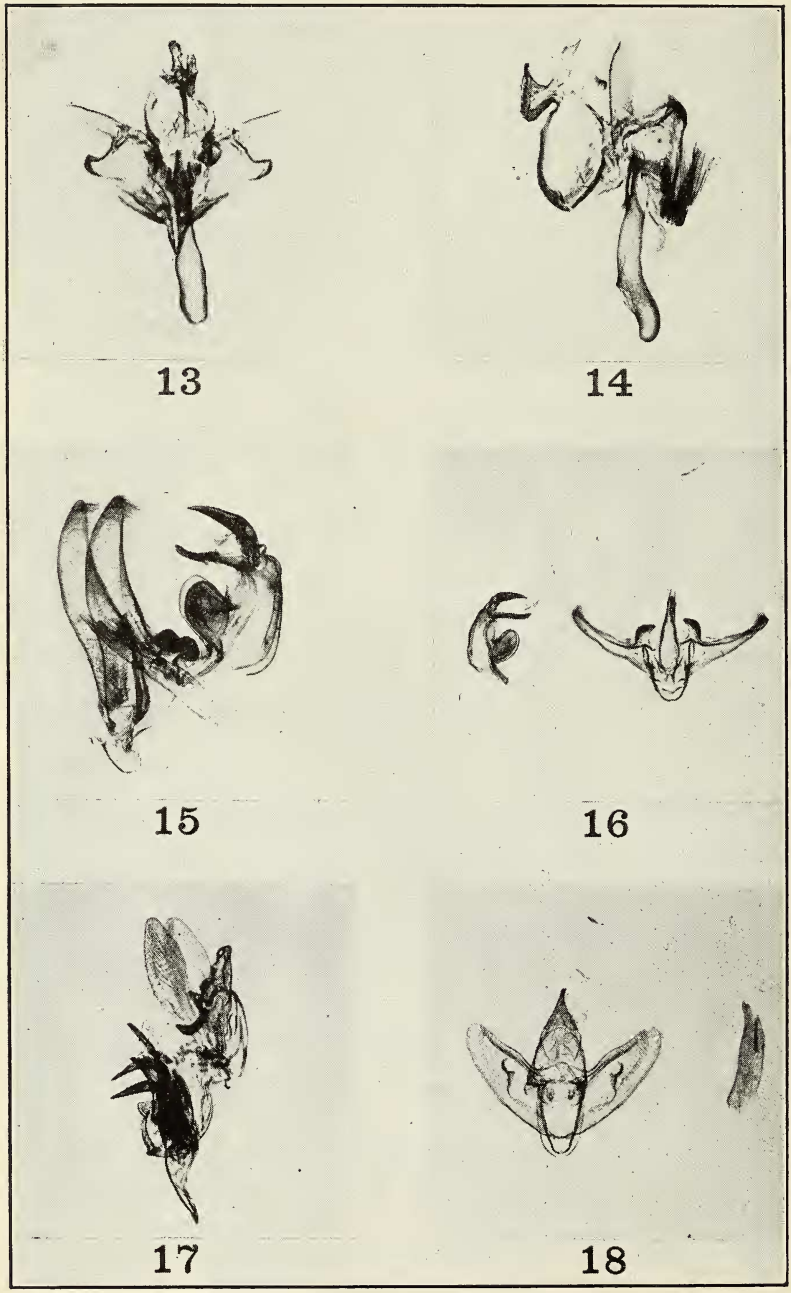
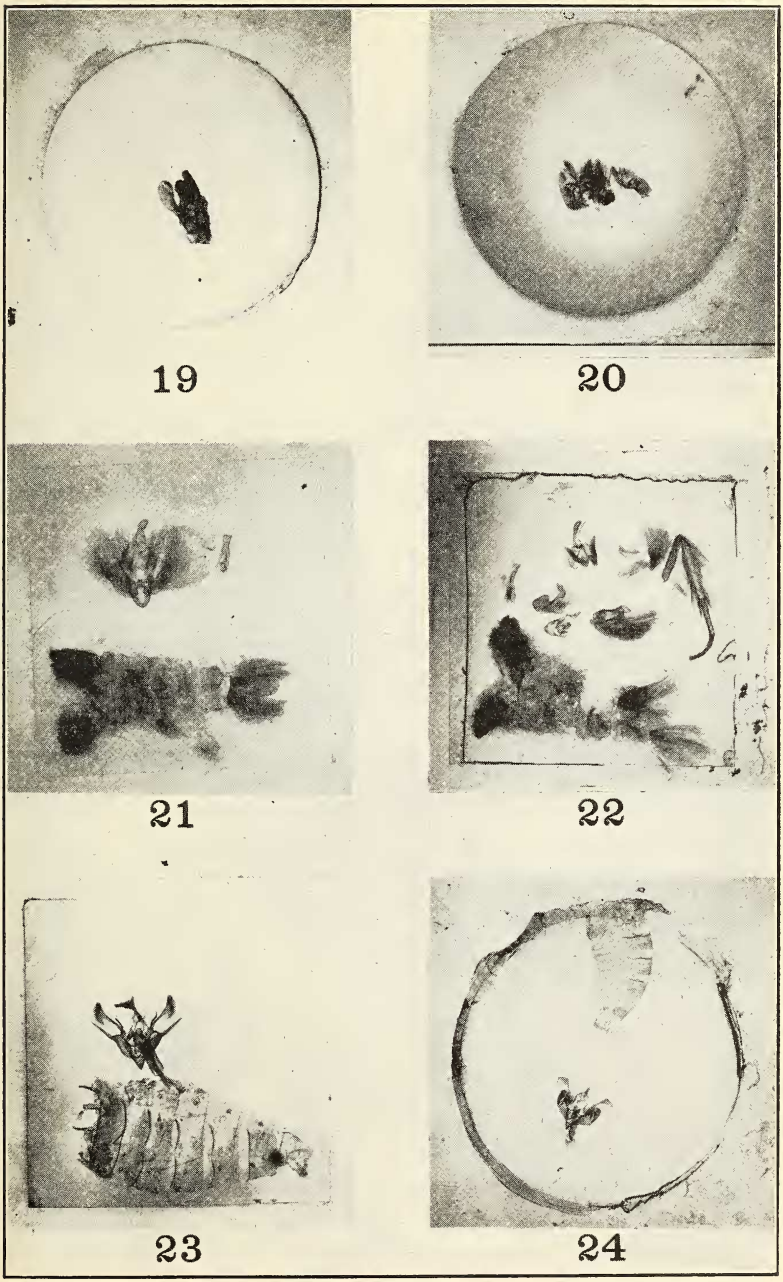


Plate V.



Explanation of Plates.

Plate II

Fig. 2. Temporary label used during preparation of abdomen for genitalic mount.

Fig. 3. A double mount slide with single label. This type is used only when the abdomen is too large to be included within the ring with the genitalia.

Fig. 4. Small label to be attached to specimen from which the abdomen has been removed. Note that the date and number correspond to the date and number on the temporary label (fig. 2).

Fig. 5. Typical arrangement of male genitalia and denuded abdomen on single mount slide.

Fig. 6. Typical arrangement of female genitalia and denuded abdomen on single mount slide. In this case the staining of the bursa copulatrix is too dark.

Plate III

Fig. 7. Phalaenidae: Ventral view of male genitalia with harpes spread and aedeagus removed, the latter in lateral aspect.

Fig. 8. Phalaenidae: Another mount of same type as shown in figure 7.

Fig. 9. Phalaenidae: Genitalia in lateral aspect with aedeagus *in situ*. Note the loss of detail here. This type of mount is essential, however, for obtaining a proper view of the uncus, gnathos, or other structures which are of great importance in some species, and which are obscured in a ventral mount.

Fig. 10. Phalaenidae: The asymmetrical type. Only experience will determine the kind of mount to be used but several, in different positions, are advisable.

Fig. 11. Phalaenidae: Ventral aspect of female genitalia. Note the detail of sclerites and bursa copulatrix brought out by proper staining. Note also the loss of detail, in the vicinity of the genital opening, due to the thickness of the mount.

Fig. 12. Phalaenidae: Same type as figure 11, except that this mount shows distortion through carelessness in handling.

Plate IV

Fig. 13. Schenobiidae: Ventral aspect with aedeagus *in situ*. Note the loss of the outline of the margin of the harpe in the unstained mount.

Fig. 14. Schenobiidae: Lateral view of the same species as that in figure 13. This view shows details of the tegumen, uncus, gnathos, and vinculum.

Fig. 15. Crambidae: Lateral view with aedeagus *in situ*.

Fig. 16. Crambidae: Combination mount of the same species as that shown in figure 15. In this type of mount the genitalia are dissected and the parts are arranged in different positions to show them to the best advantage.

Fig. 17. Gelechiidae: A three-quarter mount. This type, in which the genitalia are turned slightly to one side, gives the most detail for a single position.

Fig. 18. Geometridae: Ventral view with aedeagus removed. Symmetrical types, such as this, can be shown to the best advantage in ventral aspect.

Plate V

Examples of poorly made slides

Fig. 19. This slide is absolutely worthless for purposes of identification. All details are obscured by extraneous matter and the genitalia were mounted while wet.

Fig. 20. Another useless preparation. In this case the genitalia are broken and the details are obscured by dirt.

Fig. 21. This preparation is sufficiently well made for use in determination, but exhibits poor technic.

Fig. 22. In this case the genitalia are badly broken. This preparation can be used, but all parts show complete lack of organization.

Fig. 23. This preparation shows all necessary details but exhibits poor organization and careless technic. The abdomen is not completely denuded and contains much extraneous matter.

Fig. 24. This slide shows an example of "drifting" and sloppy technic. The essential details of the genitalia are visible and the slide is usable.